



Automated and Accurate Genome Annotation with BRAKER, GALBA, & TSEBRA

Online Workshop for ERGA Community

Who we are

Gene Prediction

BRAKER

BRAKER1: RNA-Seq

BRAKER2: Proteins

TSEBRA

BRAKER3: RNA-Seq +

Proteins

Accuracy Results

Availability

GALBA

GALBA: Proteins

Accuracy Results

Availability

Annotation Quality

Genome Browsers

Descriptive Statistics

BUSCO

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Hands on Lab Session

Katharina J. Hoff

Contact: katharina.hoff@uni-greifswald.de

Katharina J. Hoff

Senior Scientist in Bioinformatics at University of Greifswald

Short CV

2022 Habilitation (Greifswald)

2009 Ph.D. Molecular Biology (Göttingen)

2005 B.Sc. Plant Biotechnology (Hanover, stays abroad: Budapest & Alnarp)

Research

- eukaryotic genome annotation, metagenomics
- best known for: **BRAKER** & other **Gaius-Augustus** software
- 31 peer-reviewed research articles with currently 5,347 citations
- ~1 Mio € grants

Teaching

- currently 3(+1) PhD students, 2 MSc students, 1 BSc student
- applied bioinformatics, programming, statistics, & data science



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Eukaryotic Gene

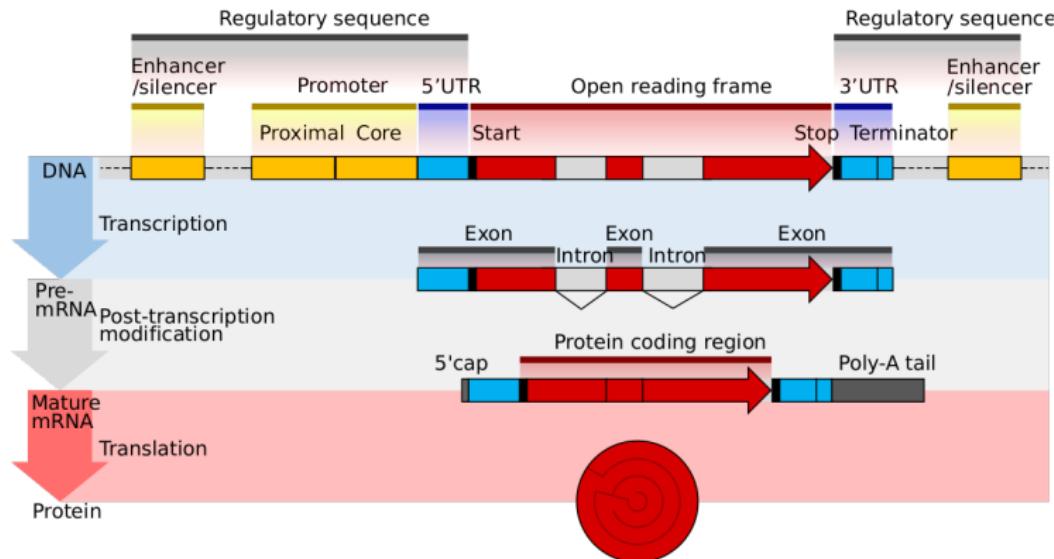
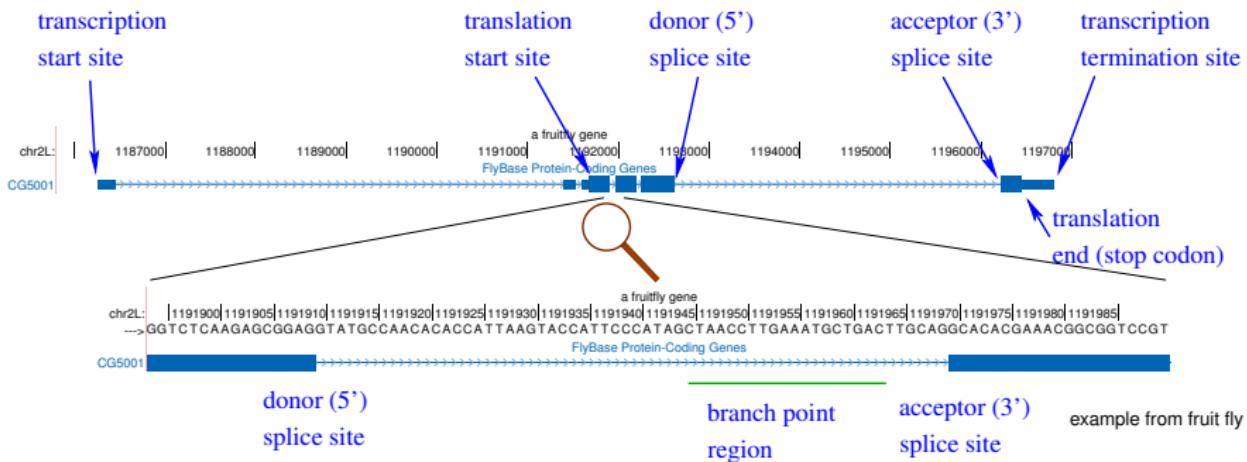


Image: Wikipedia, CC BY 4.0

Information for Genome Annotation

- genome sequence: **mathematical model**
- observed mRNA expression (parts)
- known proteins of relatives

Signals



Sequence Content

Besides the signals, **position-unspecific** frequencies of **nucleotide patterns** (e.g. k-mer frequencies) can be used to guess biological classification of longer sequence intervals.

⇒ species specific parameters ⇒ need training ⇒ need training examples



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Structural Genome Annotation Problem

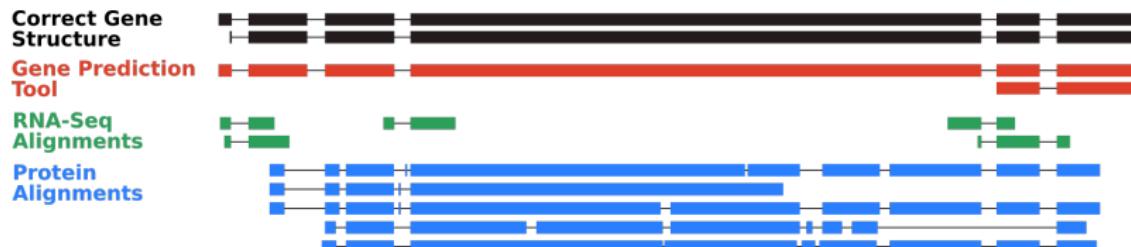
Input

- genome assembly
- extrinsic evidence, e.g. from RNA-Seq, protein sequences

Output

- protein-coding genes: CDSexon-intron structures (.gff)

Task: find and predict gene structures of protein-coding genes





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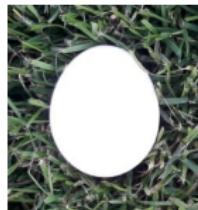
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Gene Finders Need Training Examples



Genes
(Parameter training)

Genes
(Prediction)



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Image: credits to DALL-E2, modified by human



Katharina J. Hoff



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The BRAKER Team

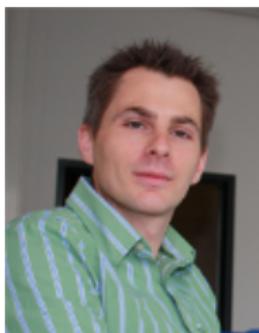
University of Greifswald & Georgia Tech University



Lars Gabriel



Alexandre Lomsadze, Katharina Hoff, Tomáš Brůna



Mario Stanke



Mark Borodovsky

Also: Simone Lange, Matthias Ebel, Hannah Thierfeldt, Anica Hoppe



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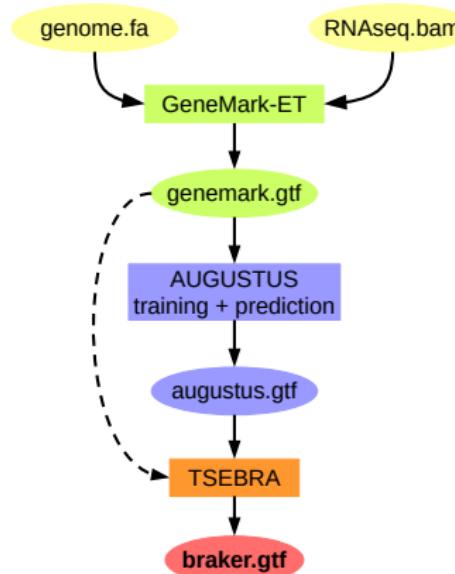
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BRAKER1: Unsupervised RNA-Seq-Based Genome Annotation with GeneMark-ET and AUGUSTUS FREE

Katharina J. Hoff ✉, Simone Lange, Alexandre Lomsadze, Mark Borodovsky ✉, Mario Stanke

Bioinformatics, Volume 32, Issue 5, 1 March 2016, Pages 767–769,

<https://doi.org/10.1093/bioinformatics/btv661>



- spliced alignments of RNA-Seq are used by GeneMark-ET and AUGUSTUS
- 1,157 citations (Google Scholar)

Whole-Genome Annotation with BRAKER

Katharina J. Hoff, Alexandre Lomsadze, Mark Borodovsky, and Mario Stanke

in Kollmar M. (eds) Gene Prediction. Methods in Molecular Biology, vol 1962. Humana, New York, NY, 2019

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GeneMark-ET uses RNA-Seq for Training

Anchors from RNA-Seq for training

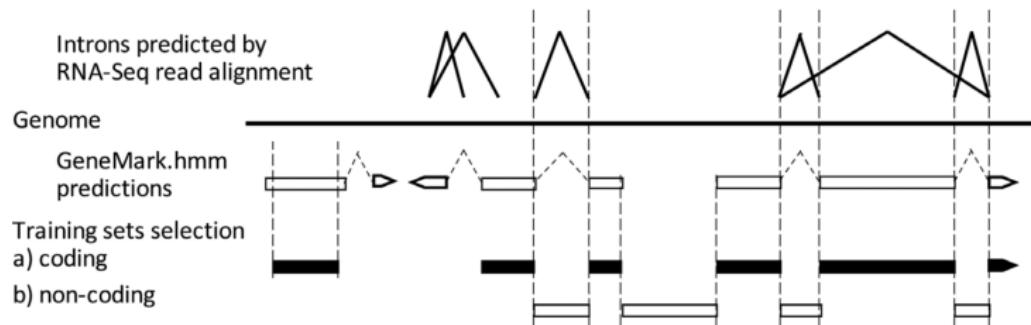


Figure 3. Selection of elements of training set in GeneMark-ET for the next iteration. The new training set of protein-coding regions is comprised from exons with at least one 'anchored splice site' as well as long exons predicted *ab initio* (>800 nt).

- employs unsupervised training
- includes in training introns and exons anchored by mapped RNA-Seq reads
- does not require RNA-Seq reads assembly
- does not use RNA-Seq information in the *prediction* step



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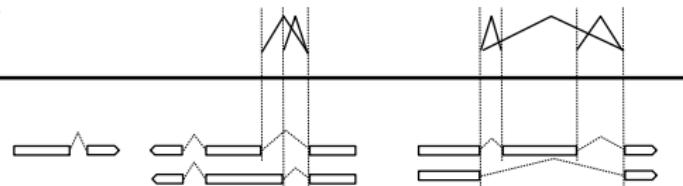
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AUGUSTUS uses RNA-Seq for Prediction

Introns predicted by RNA-Seq read alignment

Genome

AUGUSTUS gene predictions with "hints" from RNA-Seq



- requires “prior data” for training
- uses intron information from RNA-seq for *prediction*
- no RNA-Seq assembly required



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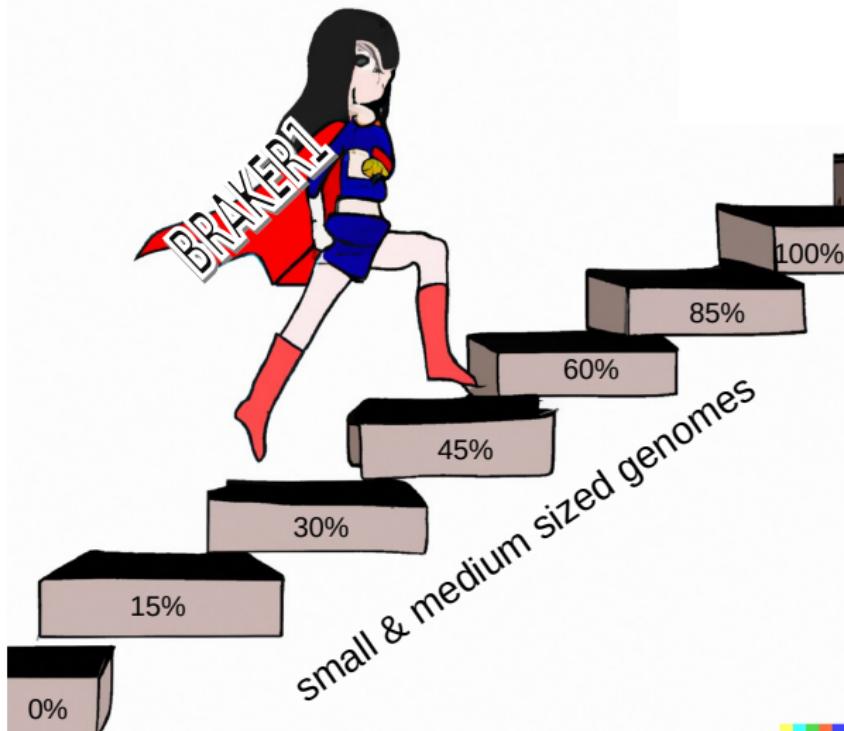
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BRAKER1 Gene F1 Accuracy

Image: credits to DALL-E2, human modification





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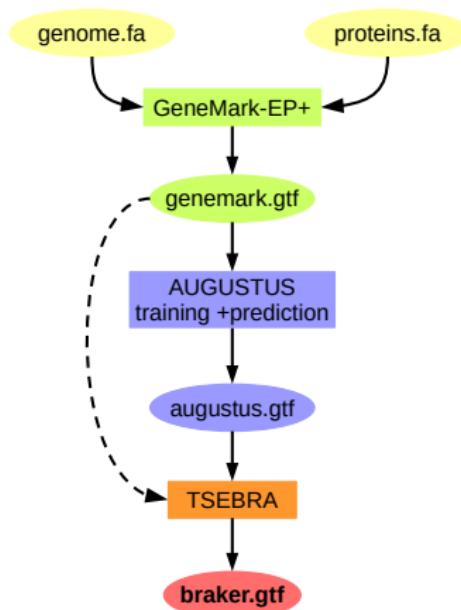
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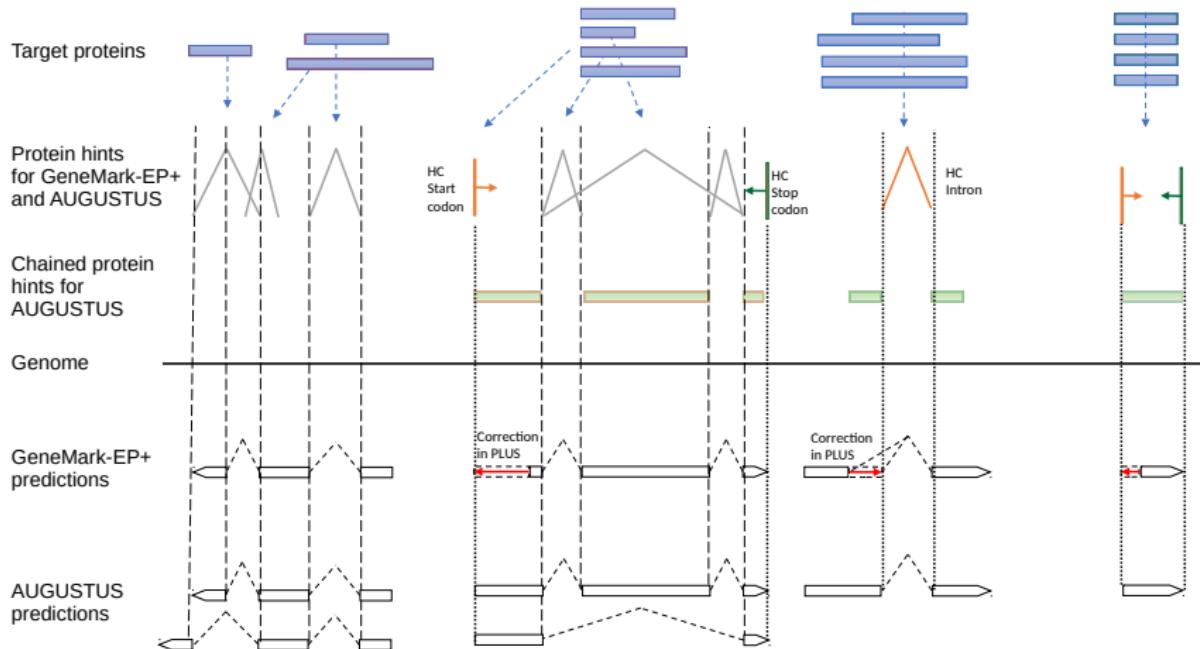
BRAKER2: automatic eukaryotic genome annotation with GeneMark-EP+ and AUGUSTUS supported by a protein database

Tomáš Brůna^{1,†}, Katharina J. Hoff^{2,3,†}, Alexandre Lomsadze⁴, Mario Stanke^{2,3,‡} and
Mark Borodovsky^{2,4,5,*;‡}



- spliced alignments of a large number of proteins
- 428 citations (Google Scholar)

Evidence Usage by GeneMark-EP+ & AUGUSTUS During Prediction





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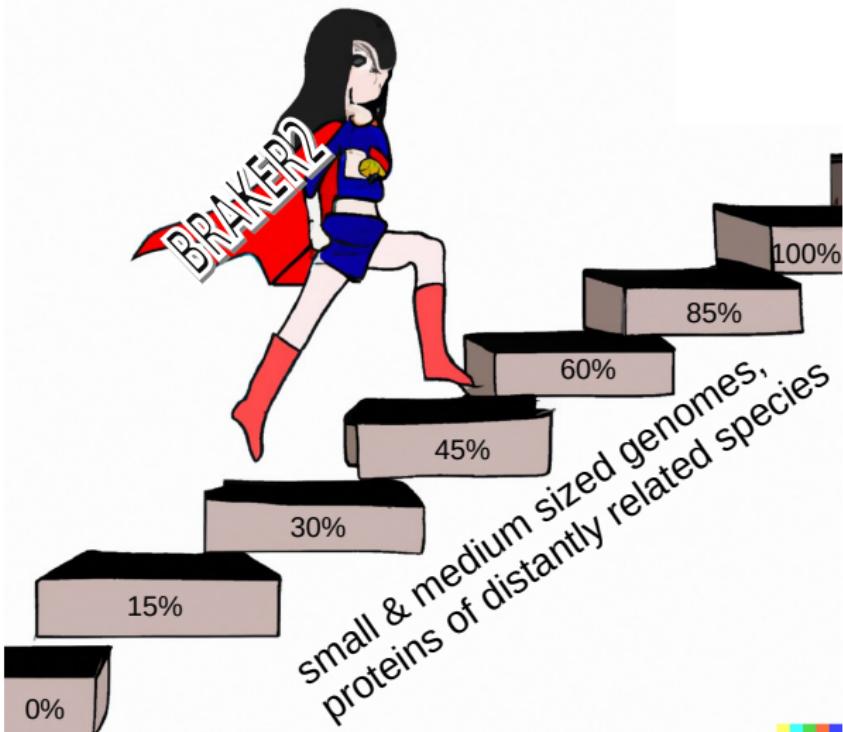
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BRAKER2 Gene F1 Accuracy

Image: credits to DALL-E2, human modification



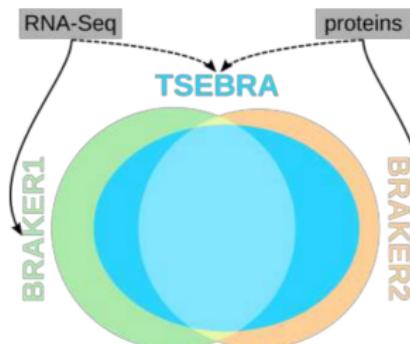
SOFTWARE

Open Access



TSEBRA: transcript selector for BRAKER

Lars Gabriel^{1,2}, Katharina J. Hoff^{1,2}, Tomáš Brůna³, Mark Borodovsky^{4,5} and Mario Stanke^{1,2*} 



- run both BRAKER1 and BRAKER2
- combine & increase accuracy
- 34 citations (Google Scholar)

Who we are

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TSEBRA

BRAKER3: RNA-Seq +
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Accuracy Results

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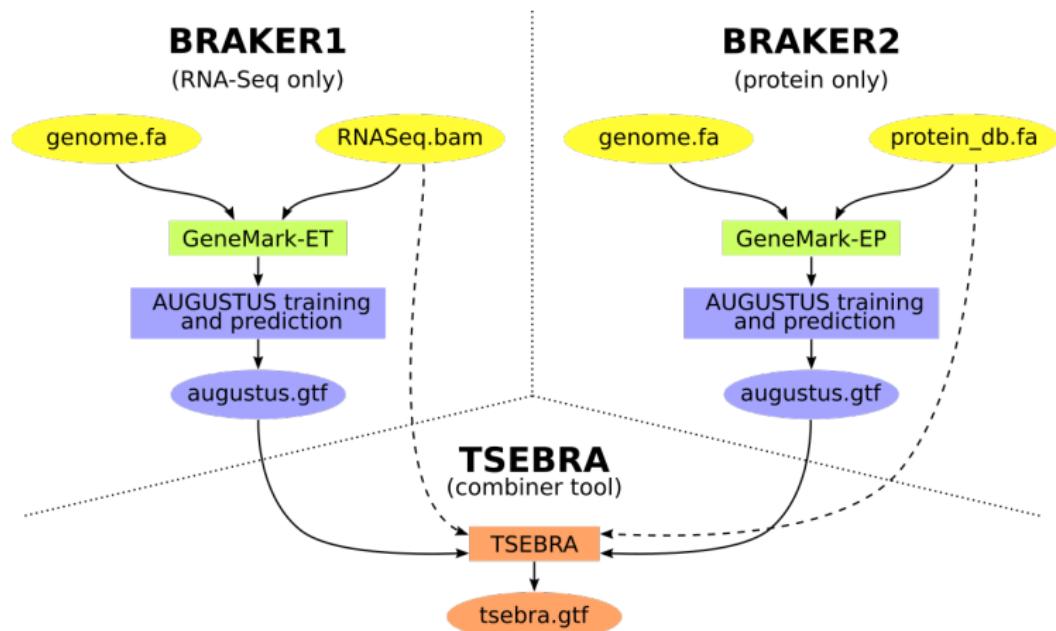
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BRAKER1 + BRAKER2 → TSEBRA



Who we are

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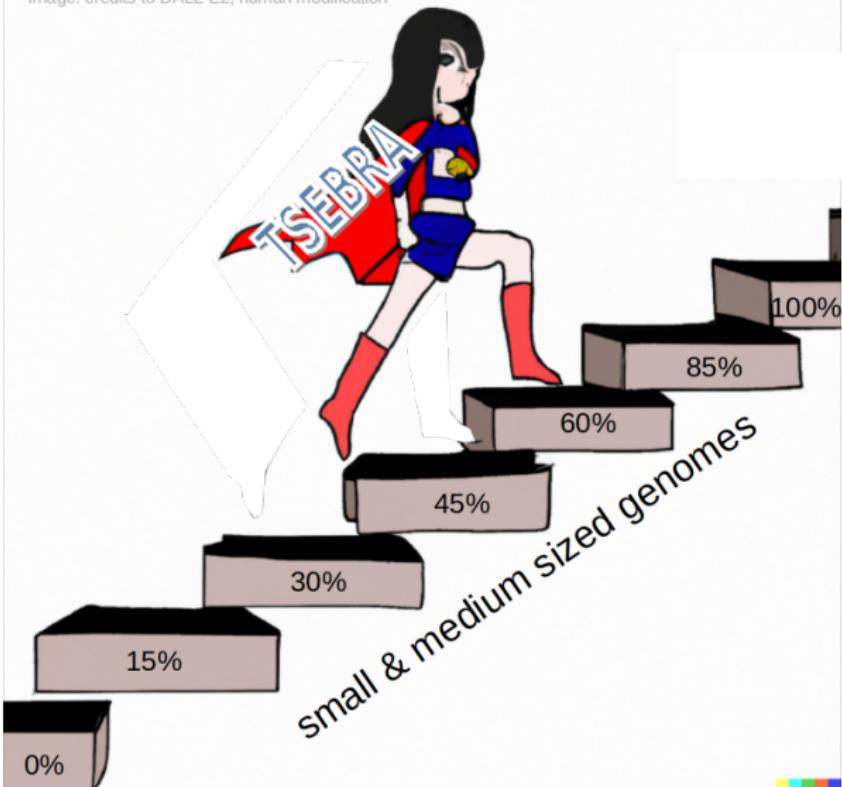
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TSEBRA: BRAKER1 + BRAKER2 Gene F1 Accuracy

Image: credits to DALL-E2, human modification





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BRAKER3: RNA-Seq +
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BRAKER3 Gene F1 Accuracy - Climbing the Top



Image: credits to DALL-E2, human modification





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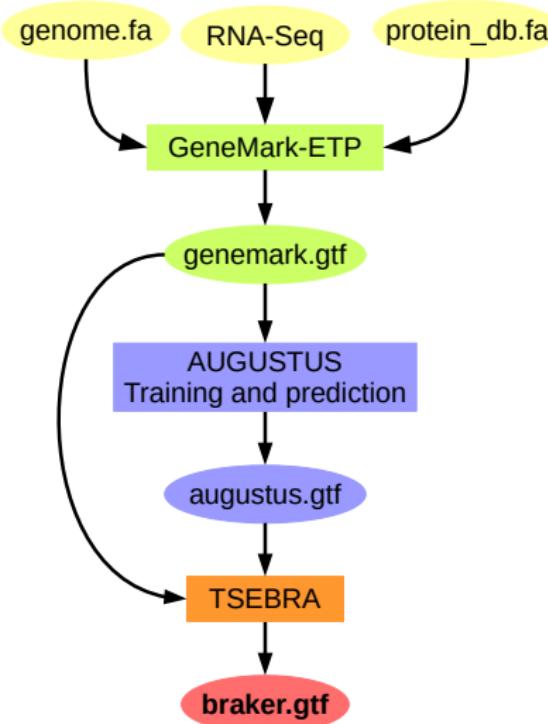
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BRAKER3: Using RNA-Seq and Protein Evidence with GeneMark-ETP, AUGUSTUS and TSEBRA



- spliced aligned and **assembled** RNA-Seq
- large protein database
- combines GeneMark-ETP and AUGUSTUS gene sets with TSEBRA

Manuscript by Lars Gabriel et. al in preparation



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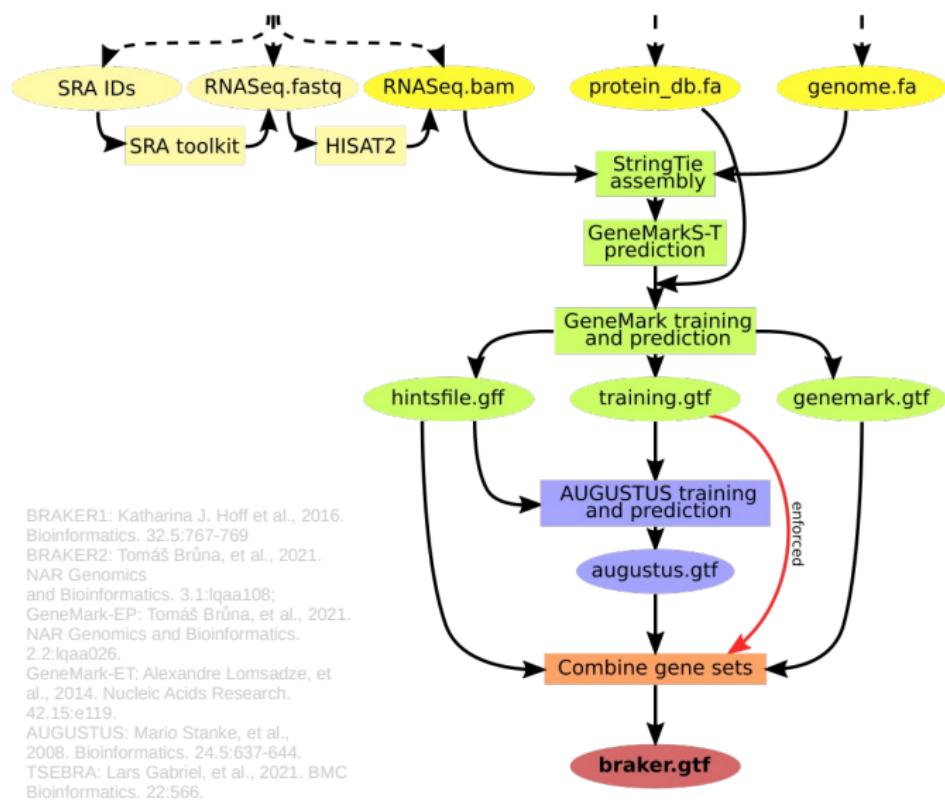
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BRAKER3 Experiments

Experiments

Accuracy assessment using genome-wide predictions in 6 species:

Species	Genome Size (Mb)	# Genes in Annotation
<i>Arabidopsis thaliana</i> (thale cress)	119	27,444
<i>Caenorhabditis elegans</i> (nematode)	100	20,172
<i>Drosophila melanogaster</i> (fruit fly)	137	13,928
<i>Gallus gallus</i> (chicken)	1,040	17,279
<i>Mus musculus</i> (mouse)	2,650	22,378
<i>Solanum lycopersicum</i> (tomato)	772	33,562

Accuracy metrics

Specificity [Sp]: Percentage of correctly found genes/transcripts/exons in the **predicted gene set**.

Sensitivity [Sn]: Percentage of correctly found genes/transcripts/exons in the **reference annotation**.

Harmonic Mean [F1]:
$$\frac{2 \cdot \text{Sensitivity} \cdot \text{Specificity}}{\text{Sensitivity} + \text{Specificity}}$$

INPUT

GeneMark-ETP

AUGUSTUS

TSEBRA

OUTPUT

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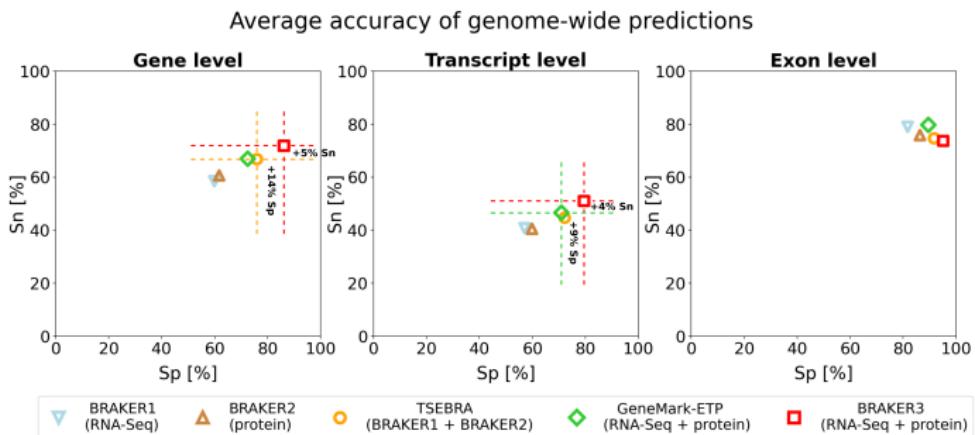
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BRAKER3 Accuracy in Small Genomes



Species: *D. melanogaster*
A. thaliana
C. elegans

Extrinsic evidence:

- paired RNA-Seq short reads
- OrthoDB v.10 clade partitions
(order excluded)

INPUT

GeneMark-ETP

AUGUSTUS

TSEBRA OUTPUT



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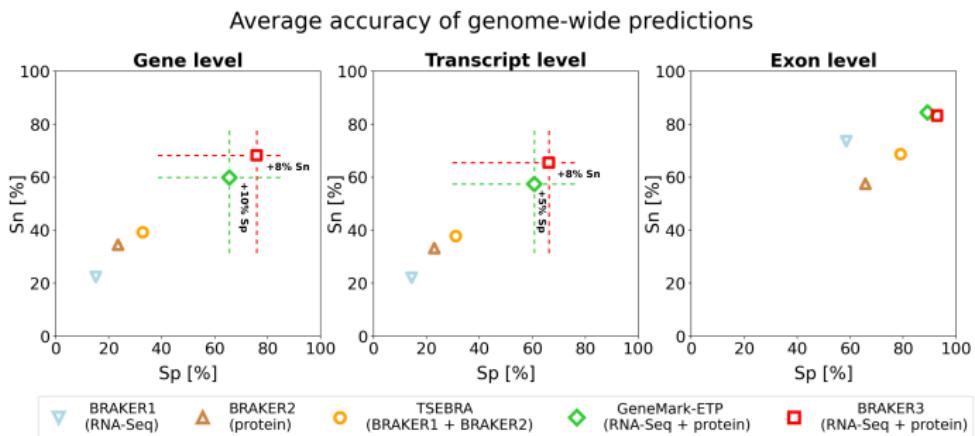
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BRAKER3 Accuracy in Larger Genomes



Species: *M. musculus*
G. gallus
S. lycopersicum

Extrinsic evidence:

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Usage & Runtime

Command line

```
braker.pl --genome=genome.fa --prot_seq=protein_db.fa \
           --rnaseq_sets_ids=RNA_ID1, RNA_ID2 \
           --rnaseq_sets_dirs=/path/to/RNASeq/
```

Runtime

- average for *A. thaliana*, *C. elegans*, *D. melanogaster*, *G. gallus*,
M. musculus, *S. lycopersicum*.
- with 48 threads:

	BRAKER1	BRAKER2	GM-ETP	BRAKER3
Runtime (h)	06:26	09:01	06:03	17:55



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Availability

GitHub

<https://github.com/Gaius-Augustus/BRAKER>

Docker/Singularity

```
singularity build braker3.sif \
    docker://teambraker/braker3:latest
```

```
singularity exec braker3.sif braker.pl [OPTIONS]
```

Licenses

- BRAKER: Artistic License
- GeneMark-ETP: License for GeneMark family software
http://topaz.gatech.edu/genemark/license_download.cgi



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The GALBA Team

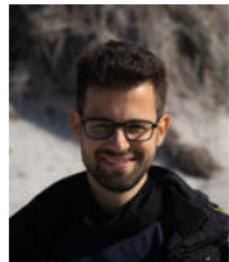
DOE Joint Genome Institute & Harvard Medical School
& University of Greifswald



Tomáš Brůna



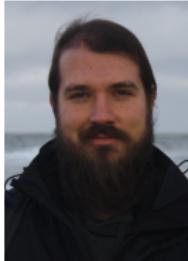
Heng Li



Lars Gabriel



Natalia Nenasheva



Matthias Ebel



Mario Stanke



Katharina Hoff

Also: Daniel Honsel, & Steffen Herboldt

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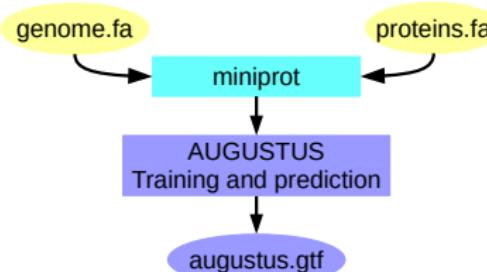
Genome analysis

Protein-to-genome alignment with miniprot

Heng Li  ^{1,2}

"Miniprot is a fast protein-to-genome aligner comparable to existing tools in accuracy. Its primary use case is to assist gene annotation."

GALBA



Do we need another pipeline?

- no RNA-Seq, large genome
- proteins of few or 1 reference species
- fully open source

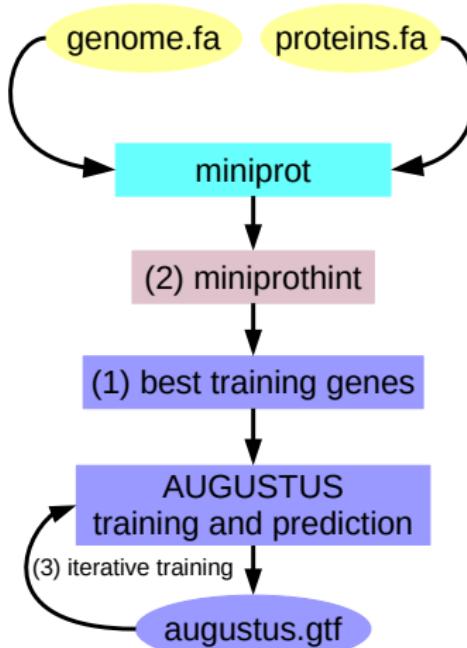
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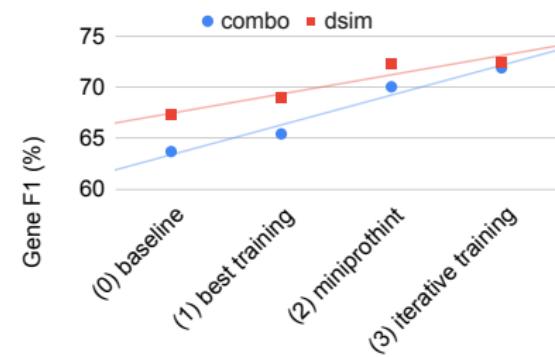
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GALBA: Proteins of Closely Related Species



Development steps in *D. melanogaster*



Donor proteins from

dsim *D. simulans*

combo *D. ananassae*,
D. pseudoobscura,
D. willistoni,
D. virilis,
D. grimshawi

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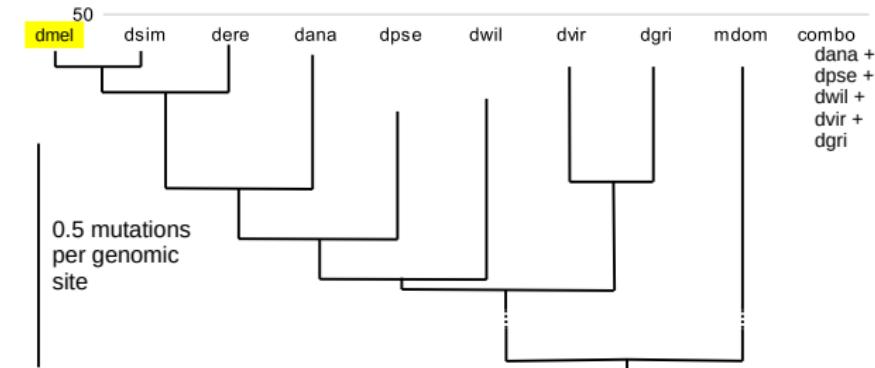
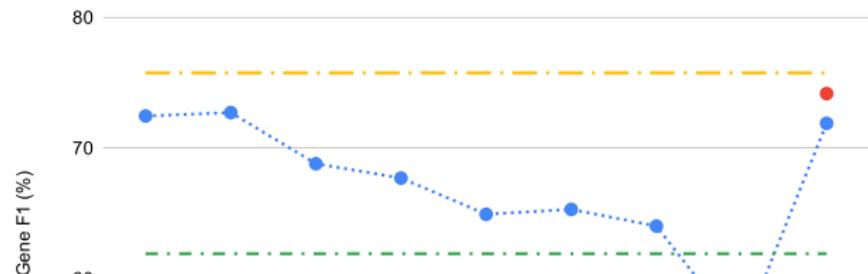
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Accuracy of GALBA with Different Protein Donors

Drosophila melanogaster

● GALBA ● BRAKER2 combo — BRAKER2 ODB10 species excl.
— BRAKER2 ODB10 order excl.





Who we are

Gene Prediction

BRAKER

BRAKER1: RNA-Seq

BRAKER2: Proteins

TSEBRA

BRAKER3: RNA-Seq +
Proteins

Accuracy Results

Availability

GALBA

GALBA: Proteins

Accuracy Results

Availability

Annotation Quality

Genome Browsers

Descriptive Statistics

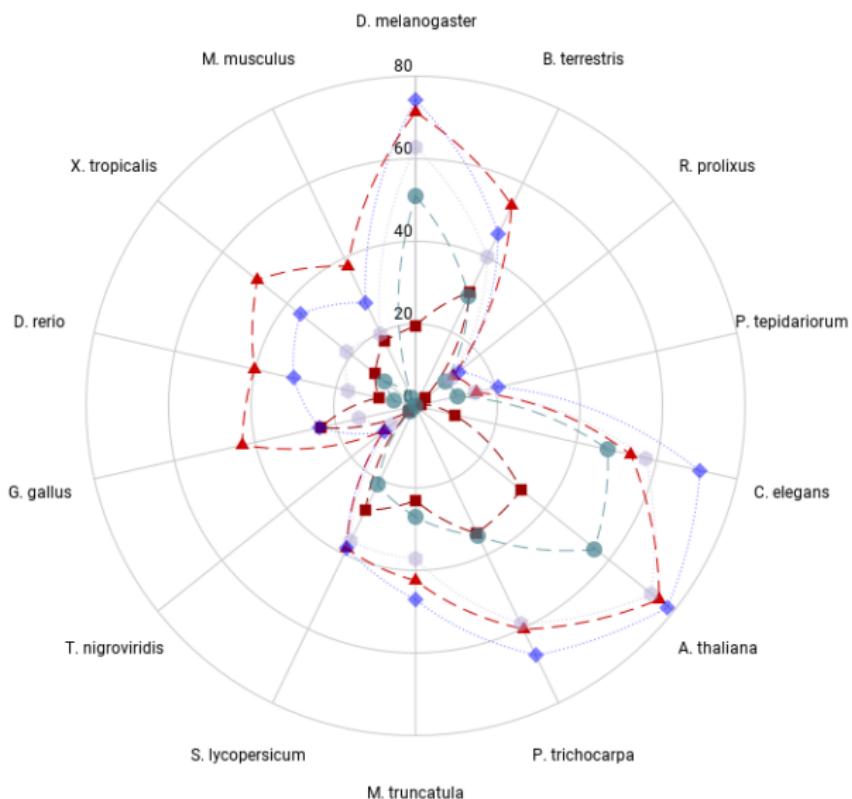
BUSCO

OMark

Hands on Lab Session

Gene F1 of GALBA in 14 Species

■ miniprot ▲ GALBA ● GeneMark-ES □ GeneMark-EP △ BRAKER2



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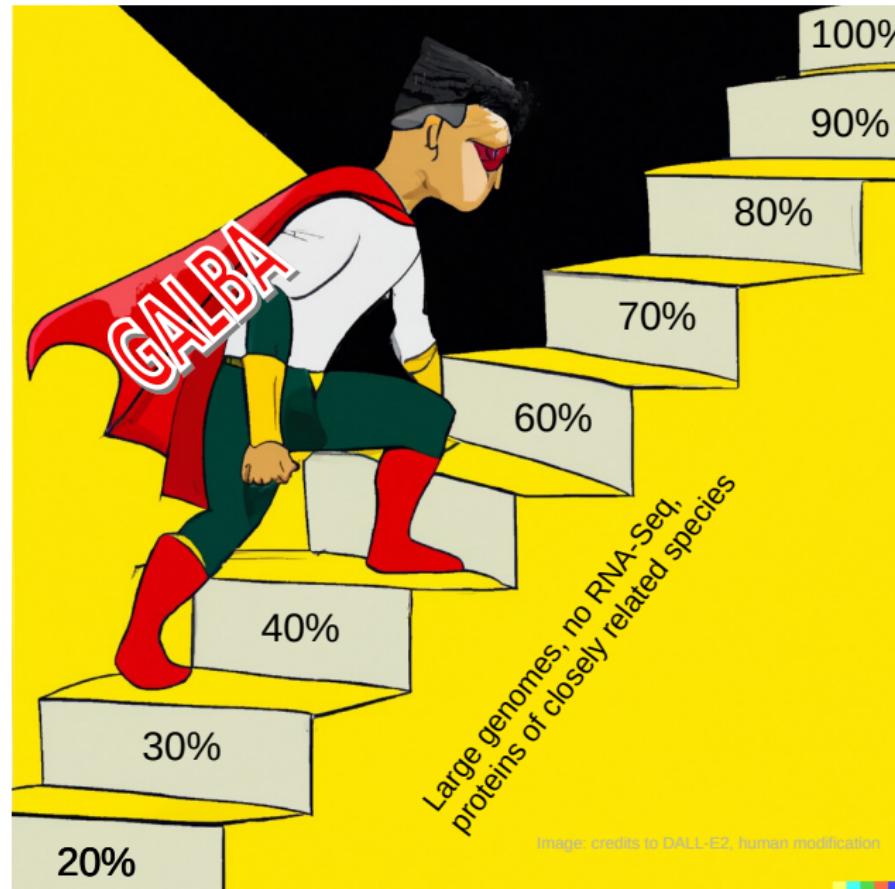
GALBA

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Hands on Lab Session

GALBA: Gene F1 Accuracy





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Availability

GitHub

<https://github.com/Gaius-Augustus/GALBA>

Docker/Singularity

```
singularity build galba.sif \
    docker://katharinahoff/galba:latest

singularity exec galba.sif galba.pl [OPTIONS]
```

Licenses

- GALBA: Artistic License
- miniprot: MIT License



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Did We Do a Good Job?





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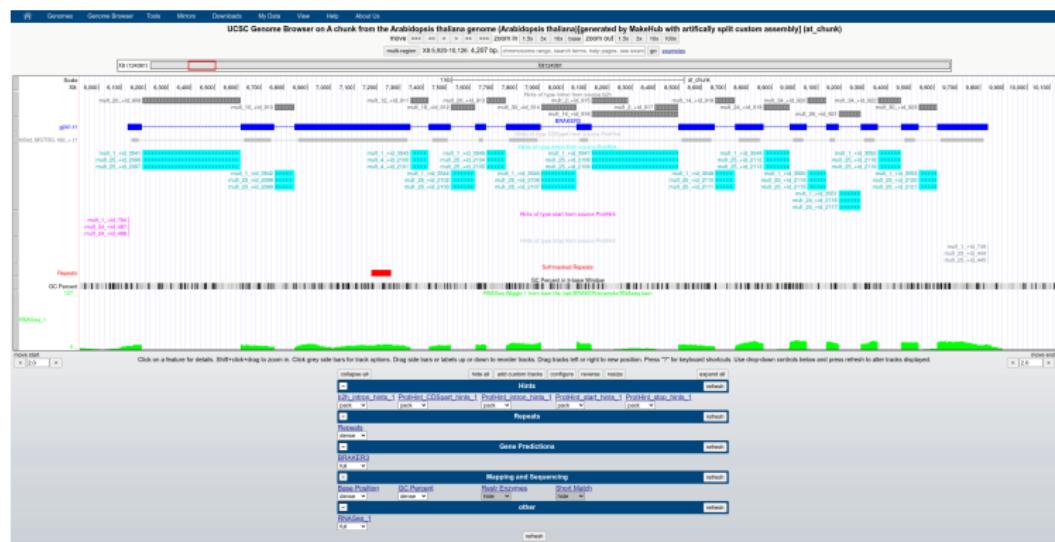
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Genome Browsers

Visualize your Annotation in Context with Evidence

- UCSC Genome Browser, MakeHub
- JBrowse
- ...





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Hands on Lab Session

Describe Your Annotation

- number of genes
- number of transcripts
- ratio of mono-exonic to multi-exonic genes
- median number of exons per transcript
- maximal number of exons per transcript
- median transcript length
- ...

If possible, compare to annotated close relatives.
Consider effect of individual annotation pipelines.



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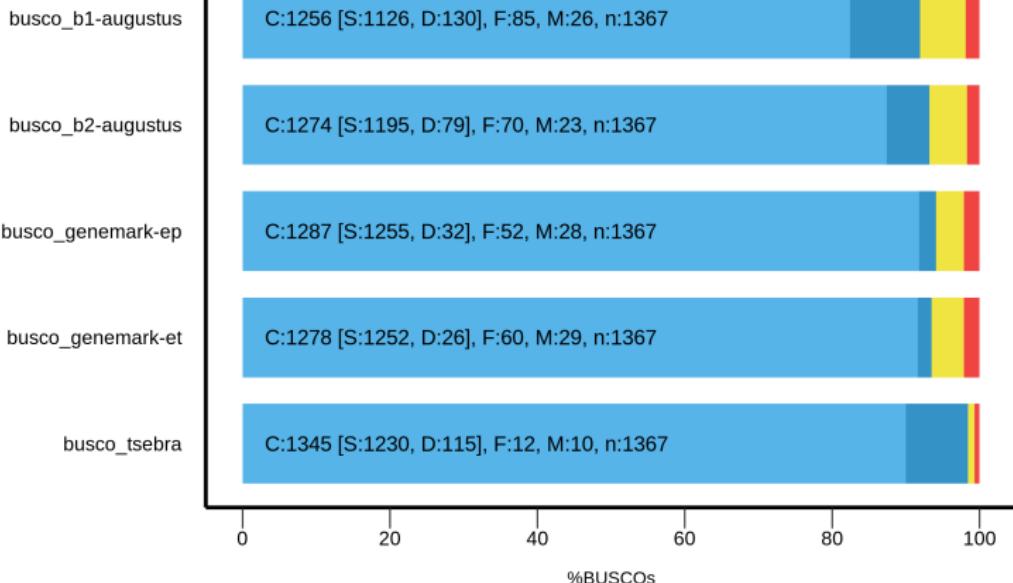
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BUSCO: Sensitivity in Clade-Specific Conserved Genes

BUSCO Assessment Results





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GALBA: Proteins

Accuracy Results

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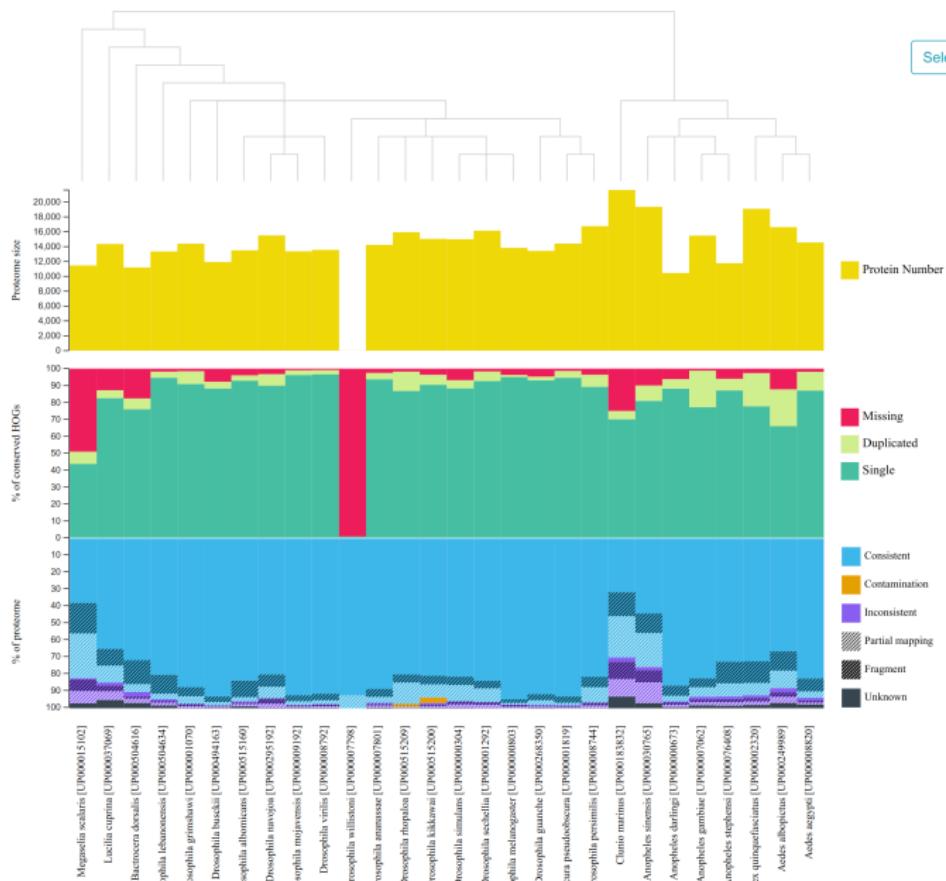
BUSCO

OMArk

Hands on Lab Session

OMArk: Sensitivity, Contaminations, & More

Select Taxon ▾





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BUSCO

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Hands on Lab Session

After a break:

- 1 Activate your VPN connection
- 2 Open Chrome
- 3 Navigate to
<https://apphub.wolke.uni-greifswald.de/>
- 4 Login
- 5 Start Jupyter -> Bioinformatics with 6 cores & 10 GB RAM



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